INTRODUCTION:
Parathyroid hormone (1-34) is a well known drug for treatment of many bone disorders including osteoporosis and osteoarthritis. The recent reports suggested that early stage administration of PTH(1-34) can suppress papain induced osteoarthritis in rat. But, the treatments requires the administration of drug once in 3 days or alternative days, which makes more sufferings and inconvenient for patient undergoing treatment. To reduce the patient suffering, a suitable drug carrier is required to controlled release of PTH(1-34). We hypothesized that the PLGA encapsulation can achieve the controlled release of PTH(1-34). In this study, we prepared the PTH(1-34) encapsulated PLGA microspheres and studied their surface, size, sustained release kinetics and bioactivity.

METHODS:
Fabrication of PLGA encapsulated PTH(1-34) microspheres: The microspheres were fabricated by the w/o/w double emulsion technique(Figure.1). We used the two different composition of PLGA in this study PLGA(50:50) and PLGA (65:35). The surface morphology and size of the PLGA microspheres were evaluated by the scanning electron microscopy (SEM) and particle size analyzer, respectively. The toxicity range of PTH(1-34) was tested by MTT analysis on treated with MC3T3-E1 cells. The PTH (1-34) release kinetics: 10 mg of microsphere were taken in 1 mL of PBS and kept in three different temperatures 4, 25, 37 °C. Everyday, 700 µL of solution was collected and replaced with equal amount of fresh PBS. The concentration of PTH(1-34) were calculated using the ELSA kit. The encapsulation efficiency of PTH(1-34) in microsphere was determined by ELISA. The bioactivity of released PTH(1-34) were measured by calculating the expression of cAMP from released PTH(1-34) treated MC3T3-E1 cells using ELISA kit. At every indicated time interval, cells were collected for further experimental analysis. Statistical analyses were performed using Student’s t -test, with p values below 0.05 being considered significant.

RESULTS:
The SEM observation emphasize that the surface of the PLGA microspheres were smooth and consistent through the degradation. The particle analyzer data showed that the mean average size of the microspheres were 45-127 µm (Fig 1,2a). The release kinetic data showed that PLGA(65:35) microspheres released the PTH(1-34) for 3 weeks with desired concentration of 1X10⁻⁸ M (Fig. 3) at 37 °C. In comparison with PLGA(50:50) the PLGA(65:35) showed the consistent release profile over 28 days at physiological condition (37 °C). The bioactivity data suggest that the released PTH(1-34) from microspheres at 1 and 3 days were increased the cAMP expression on treated MC3T3-E1 cells.

DISCUSSION:
The more suitable size of the microparticle for sustained release in articular injection is 35-105 um. Our double emulsion technique formed the appropriate microparticle size 45-127 µm. The formed PTH encapsulated PLGA microsphere released the desired concentration of PTH(1-34) for 3 weeks. These studies illustrate the feasibility of achieving controlled local delivery of PTH(1-34) and maintain their constant concentration by PLGA encapsulation method. Therefore, the PTH(1-34) encapsulated PLGA microsphere may be a potential carrier for PTH(1-34) delivery system.